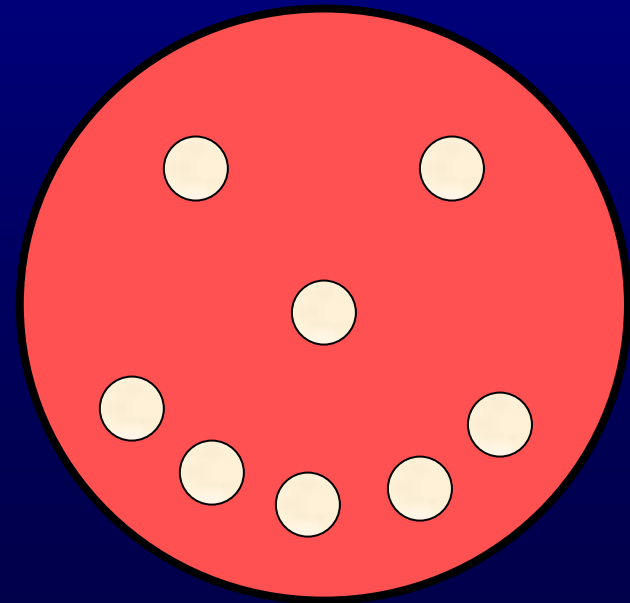


Antimicrobial Susceptibility Testing (AST): Current Issues & Implications for Public Health

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At the conclusion of this talk, you will be able to.....

- ◆ Discuss **current issues** in antimicrobial susceptibility testing and antimicrobial resistance.
- ◆ List current **sources for information** on antimicrobial susceptibility testing.
- ◆ Describe the **effects** of these issues on **public health**.



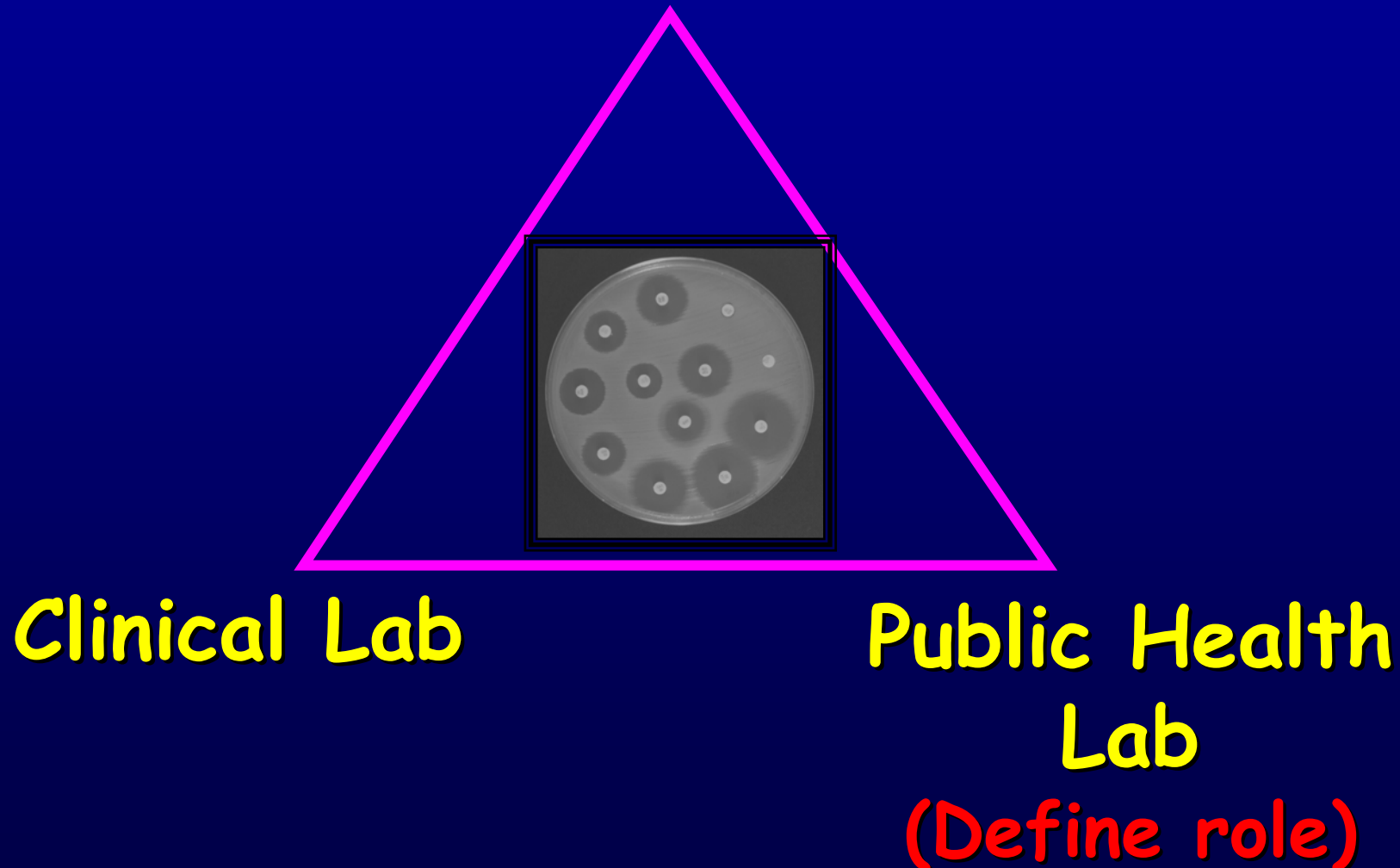
Current Issues in AST

- ◆ Identify **methods** to detect and report “emerging resistance”
 - Reliable
 - Practical for clinical laboratories
- ◆ **Communicate** AST and reporting issues to clinical labs
- ◆ **Assess performance** of clinical labs in detecting “emerging resistance”

Communicate AST & Reporting Issues to Clinical Labs

- ◆ **Primary resources** for continuing education
 - Workshops / seminars
 - Internet
 - Publications
- ◆ **CDC / NLTN efforts**
 - MASTER website
(www.phppo.cdc.gov/dls/master/default.asp)
 - AST CD ROM (available late 2002)
 - NLTN workshops, teleconferences, other

Detect / Report Emerging Resistance



Public Health Lab as A Resource for Clinical Labs in AST & Reporting

- ◆ Performs **AST** (confirms unusual results)
- ◆ Answers **questions**
 - Identifies other resources, when needed
- ◆ Provides **news** “bulletins”
- ◆ Provides **CE**

Much Variability Among PH Labs!

Clinical Lab Need

Knowledge of “resources” for AST
& reporting in their community
....particularly when a “real
problem” arises.



Assess Performance of Clinical Labs in Detecting Emerging Resistance

- ◆ Inspections
- ◆ Proficiency surveys
- ◆ Post CE monitoring
- ◆ (Competency assessment)



Contemporary Resistance Concerns

◆ *Staphylococcus aureus*

- MRSA – new tests
- VISA
- VRSA

◆ *Streptococcus pneumoniae*

- Reporting using new breakpoints

Contemporary Resistance Concerns (con't)

◆ *Streptococcus* spp.

- Beta *Streptococcus* Group B (in pregnancy) and clindamycin / erythromycin

◆ *Enterobacteriaceae*

- Extended-spectrum beta-lactamases (ESBLs)

NCCLS Standards - 2002

- ◆ M2-A7 Disk Diffusion
- ◆ M7-A5 MIC
- ◆ M100-S12 Tables*

***Updated annually**

www.nccls.org (610) 688-0100



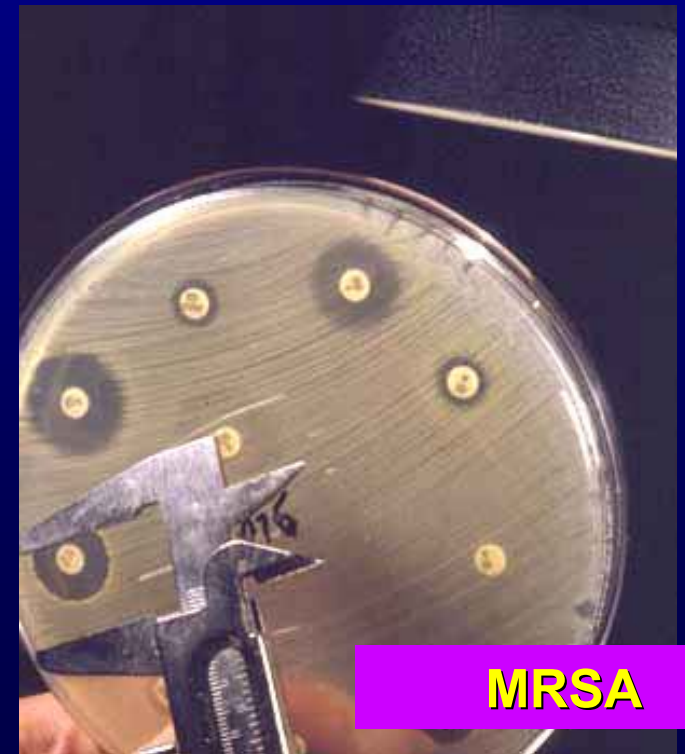
***Staphylococcus* spp.**

Organism	1st Choice Drugs	Alternative Drugs
MSS	P'ase stable penicillin (e.g., oxacillin)	a cephalosporin, vancomycin, β-lac/β-lac inhibitor combo, imipenem or meropenem, clindamycin, a fluoroquinolone
MRS	vancomycin +/- gentamicin +/- rifampin	linezolid, quin-dalfo, a fluoroquinolone, a tetracycline, trim-sulfa

The Medical Letter; 2001; 43:69.

MRSA (ORSA)

- ◆ Genetic determinant – *mecA*
- ◆ *MecA* codes for PBP2a



MRSA

MRSA Test Methods

- ◆ NCCLS disk diffusion or MIC
- ◆ Commercial method
- ◆ Agar screen
- ◆ *mecA* assay
- ◆ PBP2a assay

MRSA

“Isolates of staphylococci that are shown to carry the *mecA* gene, or that produce PBP2a, the gene product, should be reported as oxacillin resistant.”



PBP2a



Can *mecA* or PBP2a Assays be Used Alone?

- ◆ MRSA?
- ◆ MSSA?
- ◆ Are these tests consistently reliable “on the bench”?
- ◆ Are results for other drugs routinely needed?

Staphylococcus - β -Lactams

Pen	Ox	Comments
S	S	S to penicillins, cephems, carbapenems
R	S	R to β -lactamase labile pens; S to β -lactamase stable pens; S to β -lac / β -lac inhibitor combos, cephems, carbapenems
R	R	R to all β -lactams

Staphylococcus aureus

clindamycin	S
erythromycin	S
oxacillin	S
penicillin	R
vancomycin	S

“Cefazolin and other beta-lactams (except amoxicillin, ampicillin, and penicillins) are active against oxacillin-S and penicillin-R staphylococci.”

VISA* (GISA**)

- ◆ Mostly MRSA
- ◆ Vanco MIC=8 µg/ml
- ◆ Japan, USA (MI, NY, NJ, CA), Europe
- ◆ Patients previously Rx with vanco

* vancomycin-intermediate *S. aureus*

**glycopeptide-intermediate *S. aureus*
(vancomycin is a glycopeptide)

Detection of VISA

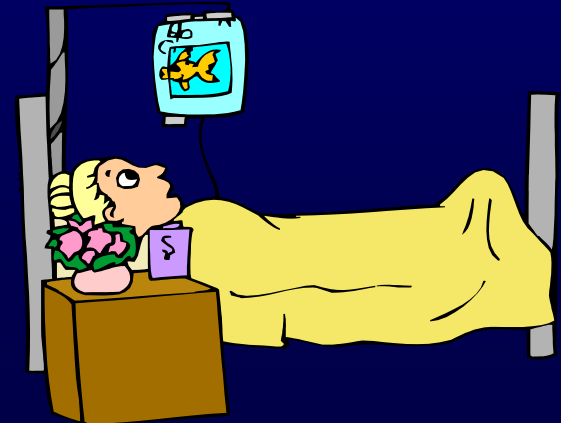
	<u>MIC (µg/ml)</u>
◆ MicroScan ON	8
◆ Etest	6-8
◆ Sensititre	4,8
◆ Vitek	4
◆ BHI-Van (6 µg/ml)	growth
◆ MicroScan rapid	≤2, ≥ 16
◆ Disk diffusion	inadequate

Tenover, et al. 1998. JCM. 36:1020

Case Study

VISA - Pt. JB (UCLA)

- ◆ 27 y.o. referral patient
- ◆ liver transplant candidate
- ◆ Bile drainage (liver abscesses)
- ◆ 2 strains VISA



Marlowe et al. 2001. JCM. 39:2637

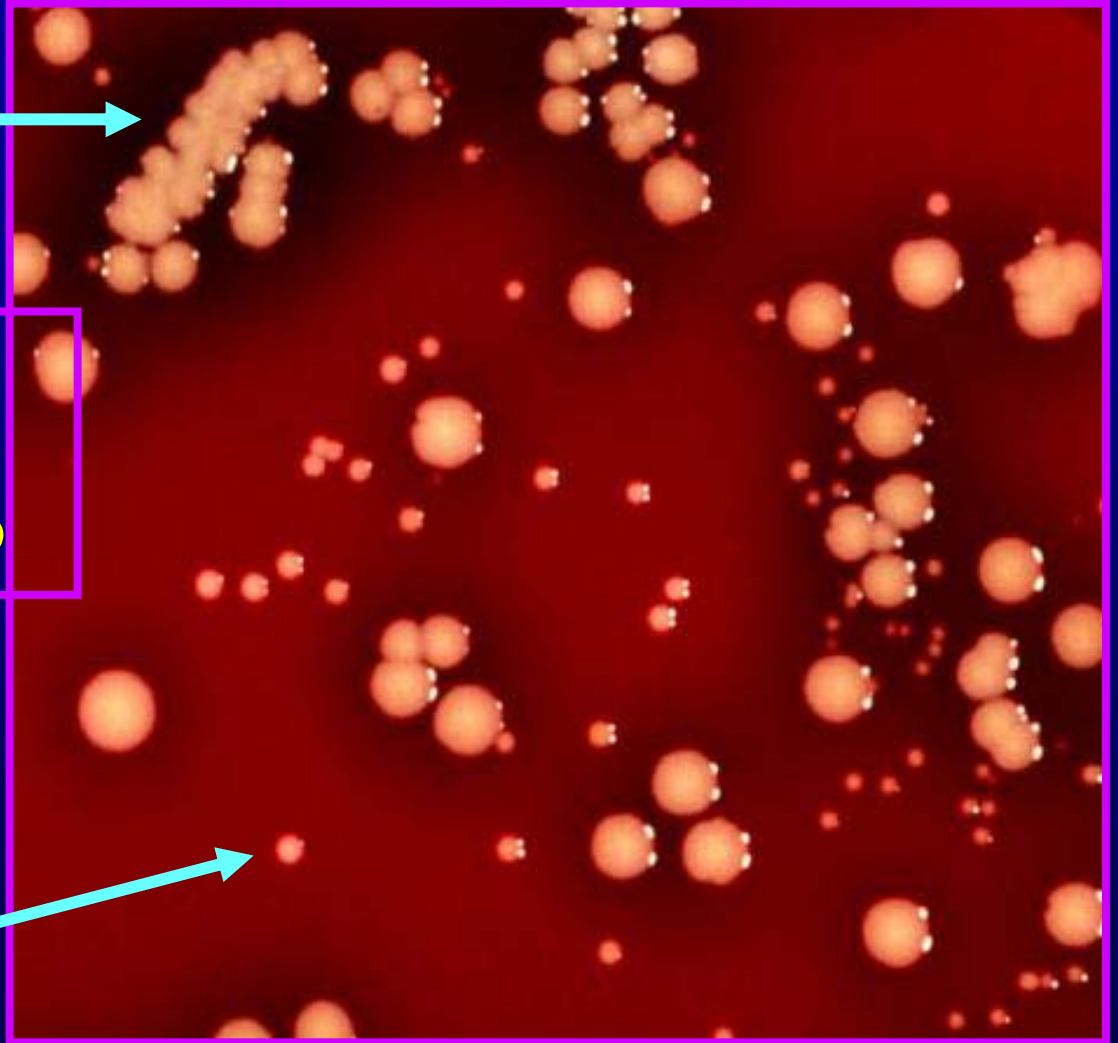
Case Study - VISA Pt. JB

Strain	<i>mecA</i>	MIC ($\mu\text{g/ml}$)	
		OX	Van
1	-	0.5	8
2	+	>16	8

MRSA →

**48h BAP
VISA - Pt. JB**

VISA →



Confirmation of VISA

◆ If **VISA** suspected:

1. Repeat ID and susceptibility tests
2. Contact your institution's infection control department
3. Contact CDC at SEARCH@cdc.gov
4. Contact your local health department
5. Save isolate

VRSA – 1st Case

- ◆ **Michigan**
- ◆ **40 y.o., diabetic on dialysis**
- ◆ **Previous catheter site with MRSA**
- ◆ **Previous vancomycin Rx (6.5 wks)**

MMWR 51:565-567, July 5, 2002

VRSA – 1st Case (con't)

- ◆ VRSA with *mecA* and *vanA* genes
- ◆ Patient also had VRE (*vanA*)
- ◆ Vancomycin MIC = 1024 µg/ml
- ◆ S to chloramphenicol, linezolid, minocycline, quin-dalfo, tetracycline, TMP-SMZ

MMWR 51:565-567, July 5, 2002

VRSA – 2nd Case

- ◆ Pennsylvania
- ◆ Chronic foot ulcer
- ◆ VRSA with *mecA* and *vanA* genes
- ◆ Vancomycin MIC = 32 µg/ml
- ◆ DD zone = 12 mm
- ◆ Van (6 µg/ml) BHI screen = growth
- ◆ S to chloramphenicol, linezolid, minocycline, quin-dalfo, rifampin, TMP-SMZ

MMWR 51:902. Oct. 11, 2002

Streptococcus pneumoniae

If pen \leq 0.06 $\mu\text{g/ml}$ (S)...

1st Choice Drugs	Alternative Drugs
penicillin G or V; amoxicillin	a cephalosporin; erythromycin; clarithromycin; azithromycin; levofloxacin, gatifloxacin, or moxifloxacin; meropenem; imipenem; trim- sulfa; clindamycin; a tetracycline

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae

If pen 0.12 - 1 µg/ml (I)...

1st Choice Drugs

**penicillin G IV or
ceftriaxone or
cefotaxime**

Alternative Drugs

**levofloxacin, gatifloxacin, or
moxifloxacin; vancomycin
clindamycin**

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae

If pen ≥ 2 $\mu\text{g/ml}$ (R)...

1st Choice Drugs

Alternative Drugs

Meningitis:

**vancomycin +
cefotaxime or ceftriaxone
+/- rifampin**

meropenem; imipenem

Other:

**as above or levofloxacin,
gatifloxacin, or
moxifloxacin**

**quinupristin/dalfopristin
linezolid**

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae

Breakpoints

◆ **Ceftriaxone and Cefotaxime**

- Originally based on treatment of **meningitis**
- NCCLS saw need for breakpoints for treatment of respiratory infections
- New non-meningitis breakpoints

◆ **Penicillin**

- Originally based on treatment of **meningitis**
- No change in breakpoints

Streptococcus pneumoniae

	MIC (µg/ml)		
	S	Int	R
Ceftriaxone or cefotaxime (meningitis)	≤ 0.5	1	≥ 2
Ceftriaxone or cefotaxime (non-meningitis)	≤ 1	2	≥ 4
Penicillin	≤ 0.06	0.12-1	≥ 2

Streptococcus pneumoniae

Penicillin

“High doses of IV penicillin (e.g. at least 2 million units every 4 hours in adults with normal renal function) or similarly ampicillin (e.g. 2 g at every 6 hours) are effective in treating pneumococcal pneumonia due to strains in the intermediate category.”

***S. pneumoniae* (CSF)**

MIC ($\mu\text{g/ml}$)

ceftriaxone	
(meningitis)	≤ 0.25 S
penicillin	≤ 0.03 S
vancomycin	0.5 S

Patients with meningitis require therapy with maximum doses of ceftriaxone.

***S. pneumoniae* (blood)**

	<u>MIC ($\mu\text{g/ml}$)</u>
ceftriaxone (meningitis)	1 I
ceftriaxone (non-meningitis)	1 S
erythro, trim-sulfa	R
levofloxacin	0.5 S
penicillin	1.0 I
vancomycin	0.5 S

Pts. with meningitis require therapy with max doses of ceftriax; High dose IV pens (e.g. at least 2 mil U every 4 h in adults with normal renal function) or amp (e.g. 2 g at every 6 h) are effective in treating pneumococcal pneumonia due to strains in the penicillin “int” category.

Beta *Streptococcus* spp.

	1st Choice Drugs	Alternative Drugs
Beta Group A, C, G	penicillin G or V	clindamycin; erythromycin; a cephalosporin; vancomycin; clarithromycin; azithromycin
Group B	penicillin G or ampicillin	a cephalosporin; vancomycin; erythromycin

The Medical Letter; 2001; 43:69.

Throat Culture

Many Group A Streptococcus

**“Group A Streptococcus
remains universally
susceptible to penicillin”**

Beta *Streptococcus* spp.

Erythromycin/Clindamycin

- ◆ Erythromycin and/or clindamycin are alternatives in penicillin-allergic patients
- ◆ **Group A** - 5-10% erythromycin-R
- ◆ **Group B** - up to 25% erythromycin-R and 15% clindamycin-R (prophylaxis in pregnant women issue)

Extended-Spectrum β -lactamases (ESBLs)

- ◆ Result from mutation of common β -lactamase genes (e.g., *bla*_{TEM-1}, *bla*_{SHV-1})
- ◆ Inactivate extended-spectrum β -lactam agents
- ◆ Genes located on plasmids (often with other **R** genes)
- ◆ Approx. 100 TEM and 30 SHV types

Bradford, P. 2001. CMR. 14:933.

Significance of ESBLs

- ◆ Cause nosocomial infections
- ◆ Serious, infections. **Treatment failures** (esp. bacteremia) when patient treated with 3rd gen. cephalosporins.

ESBLs – Testing

- ◆ **Organisms:**
E. coli, *Klebsiella* spp.
- ◆ **Screen test:**
Decreased susceptibility to extended-spectrum β -lactams
- ◆ **Phenotypic confirmatory test:**
 β -lactam activity restored by β -lactamase inhibitor (e.g. clavulanic acid)
- ◆ **Other clues:**
“S” to cephamycins; multi-R

Klebsiella pneumoniae ?ESBL

PRELIM:

	<u>MIC (µg/ml)</u>
cefoxitin	1 S
ciprofloxacin	0.5 S
imipenem	≤ 0.25 S
pip-tazo	8 S
am, cfaz, gm, T-S	R

***hold cefepime, cefotaxime, if “S”

This *K. pneumoniae* is suspicious for extended-spectrum beta-lactamase (ESBL) production; confirmatory tests pending.

ESBL

Phenotypic Confirmatory Test

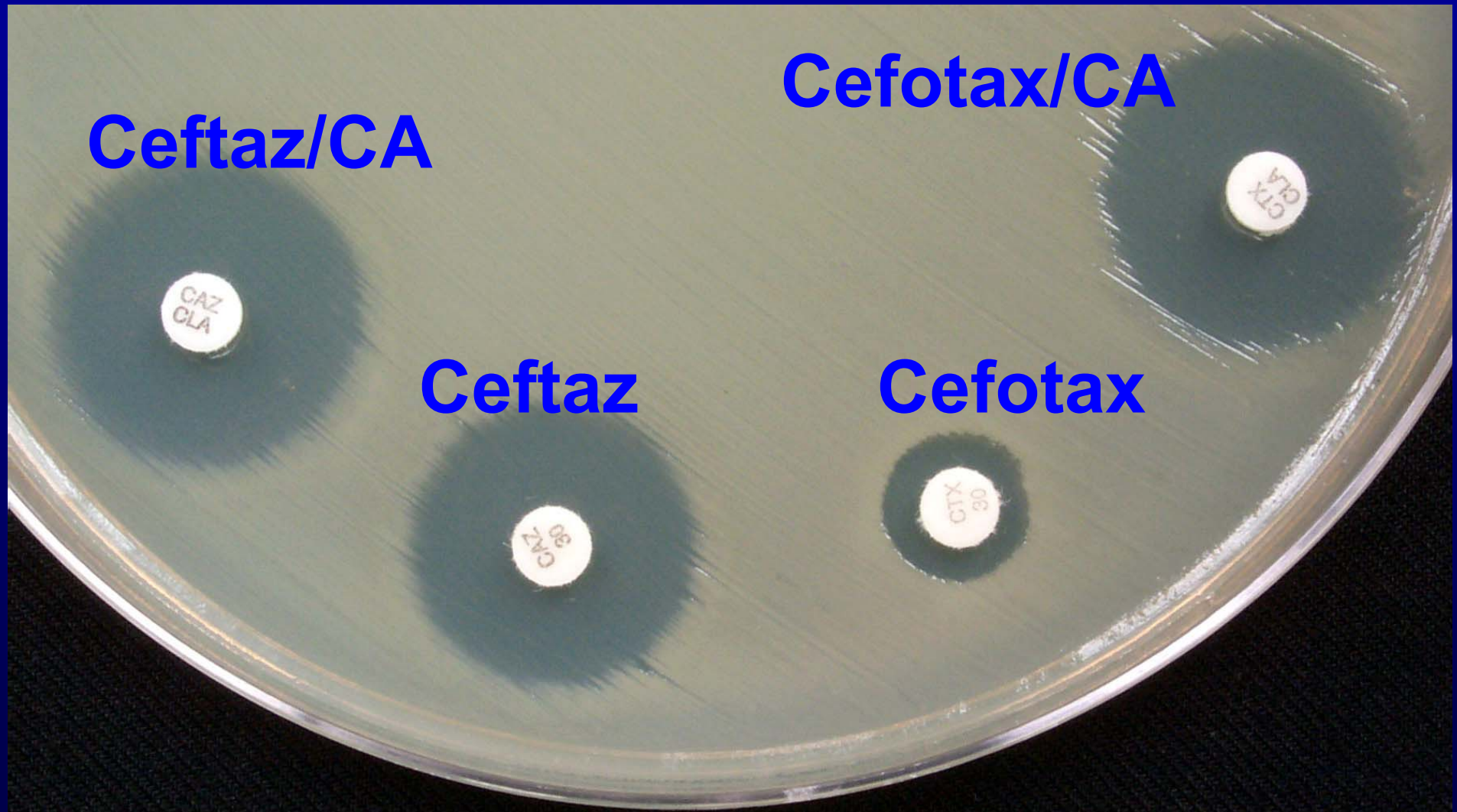
◆ Test:

- cefotaxime
- cefotaxime/clavulanic acid
- ceftazidime
- ceftazidime/clavulanic acid

◆ Results:

- clavulanic acid restores activity of cefotaxime or ceftazidime or both

ESBL Confirmatory Test



ESBL Reporting

- ◆ **Confirmed ESBLs** - report “R” for:
 - cephalosporins (not cephamycins)
 - penicillins (not β -lac inhibitor combos)
 - aztreonam

Klebsiella pneumoniae ESBL

FINAL:

MIC (µg/ml)

cefepime	2 S R
cefoxitin	1 S
cefotaxime	1 S R
ciprofloxacin	0.5 S
imipenem	0.25 S
pip-tazo	8 S
am, cefaz, gent, T-S	R

Confirmatory tests for this *K. pneumoniae* indicate unusual resistance [extended-spectrum beta-lactamase (ESBL) production]; ID consult suggested.

Issues - ESBLs

- ◆ Reporting ESBLs from urine isolates (e.g. acute cystitis)
- ◆ Changing results for other β -lactams (e.g. piperacillin-tazobactam)
- ◆ Detection of.....
 - all types of ESBLs (\cong 100 TEM and 30 SHV)
 - ESBLs present with other “R” mechanisms
 - ESBLs beyond *E. coli* and *Klebsiella* spp.
 - other β -lactamases (e.g. plasmid-mediated ampC)

NCCLS M39-A Guideline

“Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data”

Focus - clinical laboratories

Guide MDs on **empiric** therapy



% Susceptible 2001 – Exmp.

	n	Am	Cf	Ctx	Cip	Gm	Pp	T-S
<i>E. coli</i>	729	66	81	98	97	97	66	76
<i>E. cloacae</i>	144	-	-	66	95	88	65	88
<i>P. aerug</i>	221	-	-	10	79	81	76	-

CAP Checklist

MIC.21950 (2001)

- ◆ **Does the procedure manual address unusual or inconsistent antimicrobial susceptibility testing results?**
 - Testing QC strains doesn't guarantee accurate patient results
 - Unusual or inconsistent results should be investigated

Verification Rules - Exmps.

Biologically implausible or infrequent

◆ Repeat test

- amik-R + gent-R + tob-R

Enterobacteriaceae

- amp-R *E. faecalis*

- imipenem-R Enterobacteriaceae

◆ Reexamine or repeat test

- amp-S *Klebsiella* spp.

Enterobacter aerogenes

ampicillin	R
cefazolin	R
ceftizoxime	S
gentamicin	S
imipenem	R*
trimeth-sulfa	S

*** "R" likely due to drug deterioration**

Conclusions

- ◆ **Clinical laboratory detection of emerging resistance**
 - Labs must have access to current testing / reporting guidelines
 - Lab personnel must be competent in testing
 - Resources for “problems” in emerging resistance must be available to labs
- ◆ ***Staphylococcus aureus***
 - *mecA* or PBP2a tests can be used to identify MRSA
 - VISA are uncommon and may be difficult to detect
 - VRSA are rare and can be detected readily; however the resistance may be unstable

Conclusions (con't)

◆ *Streptococcus pneumoniae*

- The new NCCLS breakpoints for ceftriaxone and cefotaxime should be reported
- MICs should be performed on CSF isolates ASAP
- A comment should be added to explain the meaning of a penicillin “Int” result on non-CSF isolates

◆ *Streptococcus spp.*

- Group B streptococci from penicillin-allergic pregnant women should be tested with clindamycin and/or erythromycin

Conclusions (con't)

◆ *Enterobacteriaceae*

- *E. coli* and *Klebsiella* spp. should be tested for ESBL production
- Urine isolates associated with acute uncomplicated cystitis may not require ESBL testing
- There are currently no standard NCCLS methods for detecting ESBLs in isolates other than *E. coli* and *Klebsiella* spp.

◆ Local resistance patterns

- When possible, NCCLS M39-A should be followed for preparing a cumulative antibiogram report

Conclusions (con't)

◆ Verifying results on patient isolates

- Each laboratory should develop a written protocol for verification of unusual or atypical patient results prior to reporting them

◆ Resources

- a listing of antimicrobial susceptibility testing resources can be found on MASTER website

Additional References

Livermore, D. M., T. G. Winstanley, and K. P. Shannon. 2001. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *J. Antimicrob. Chemother.* 48:87-102.

Websites:

<http://www.cdc.gov/drugresistance/factsheets/index.htm>

CDC antimicrobial susceptibility testing fact sheets

<http://www.cdc.gov/drugresistance/>

CDC drug resistance

<http://www.cdc.gov/drugresistance/community/>

CDC antibiotic resistance

<http://www.asmusa.org/division/c/index.htm>

ASM Division C (clinical microbiology) website includes “Askit” feature

Thank you!

Multi-level

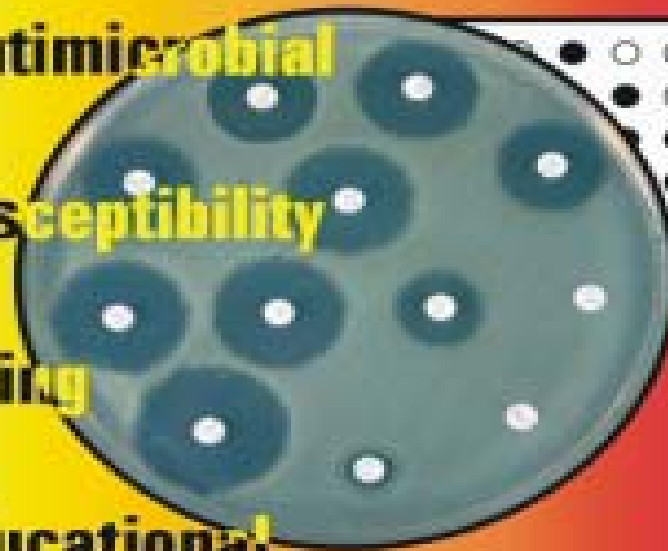
Antimicrobial

Susceptibility

Testing

Educational

Resources



www.phppo.cdc.gov/dls/master/default.asp